Genetic relationships of body composition, serum leptin, and age at puberty in gilts[1,2]

L. A. Kuehn,[3] D. J. Nonneman, J. M. Klindt, and T. H. Wise

USDA, ARS, US Meat Animal Research Center, Clay Center, NE 68933

ABSTRACT: Leptin produced by adipocytes acts through leptin receptors in the hypothalamus to control appetite and food intake and thus communicates information about degree of fatness. It is thought that a degree of body fat is required for initiation of puberty and maintenance of reproductive function in mammals. The objective of this study was to determine whether polymorphisms in the leptin (*LEP*), leptin receptor (*LEPR*), paired box 5 (*PAX5*), aldo-keto reductase (*AKR*), and pro-opiomelanocortin (*POMC*) genes were associated with age, leptin concentration, backfat as an indicator of body condition, or BW at puberty in 3 lines of gilts and to characterize genetic relationships among these traits. The first 2 lines, born in 2001, were formed by crossing maternal White Cross (Yorkshire × Maternal Landrace) gilts to Duroc (n = 210) or (lean) Landrace (n = 207) boars. The remaining line (n = 507), born in 2002, was formed by crossing progeny of the Duroc- and Landrace-sired lines. At first estrus, age, BW (BWP), and backfat (BFP) at puberty were recorded and blood was collected for leptin assays. Nine SNP were detected in candidate genes/regions: 1 in *LEP*, 3 in *LEPR*, 1 in *PAX5*, 2 in *AKR*, and 2 in *POMC*. Animals were genotyped for each of the SNP; genotypes were validated using GenoProb. The association model included fixed effects of farrowing group, covariates of SNP genotypic probabilities (from GenoProb), and random additive polygenic effects to account for genetic similarities between animals not explained by SNP. Variance components for polygenic effects and error were estimated using MTDFREML. Leptin concentrations were logarithmically transformed for data analysis. All 4 traits were moderately to highly heritable (0.38 to 0.48). Age and leptin at puberty had a significant ($P < 0.01$) genetic correlation at -0.63 ± 0.097, and the genetic correlation between BWP and age at puberty was 0.65 ± 0.083 ($P < 0.01$). Significant additive associations (a; $P < 0.05$) were detected at *PAX5* for age at puberty (a = 3.2 d) and for BFP (a = 0.61 mm). One SNP in *LEPR* was associated with leptin concentration (a = 0.31 log units; $P < 0.05$). The associations from *PAX5* correspond to a QTL peak for age at puberty detected on SSC1. Although not necessarily the causative mutation, this result implies that a QTL that can decrease age at puberty without increasing BFP and BWP at puberty may exist in this region in commercial pigs.

Key words: estrus, leptin, leptin receptor, puberty, single nucleotide polymorphism, swine

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INTRODUCTION

Gilts that achieve early puberty tend to be more fertile throughout their breeding lifetime (Nelson et al., 1990), have a shorter weaning to estrus interval (Sterning et al., 1998), and have increased retention in the breeding herd as parity increases (Foxcroft and Aherne, 2001). Age at puberty can be changed through selection (Lamberson et al., 1991), but collecting phenotypes can be labor-intensive.

During pubertal development in gilts, serum leptin concentrations increase (Qian et al., 1999), followed by increases in LH secretion and serum estrogen at puberty (Barb et al., 2000). The effect of leptin on LH secretion is associated with sexual maturation and may act as a permissive metabolic signal for initiation of puberty in the pig (Barb et al., 2005). Increases in leptin concentrations are coordinated with the onset of puberty (Maqsood et al., 2007) and fat mass associated with puberty in females (Ahmed et al., 1999), whereas leptin receptor mutant mice fail to reach puberty (Bates et

[1]Mention of trade name proprietary product or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable.

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[3]Corresponding author: Larry.Kuehn@ars.usda.gov

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Table 1. Population structure and means and SD of puberty measures

				Age at puberty, d		Leptin,[1] log ng/mL		Backfat, mm		BW, kg	
Line	Sires, n	Dams, n	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Duroc × BT[2]	12	92	210	206.7	26.5	0.608	1.078	19.71	4.55	139.5	21.2
Landrace × BT	12	82	207	208.1	23.9	0.605	1.129	19.56	4.44	129.4	17.3
4-way cross	24	202	507	203.7	24.1	0.470	1.230	17.97	3.45	125.8	17.0

[1]Base scale leptin mean values are 1.84, 1.83, and 1.60 ng/mL for Duroc-sired F_1, Landrace-sired F_1, and 4-way cross gilts, respectively. Approximate 95% confidence intervals of the data for back-transformed leptin [e^(mean ± 2 × SD)] are 0.213 to 15.863, 0.191 to 17.514, and 0.137 to 18.728 ng/mL for each respective line.

[2]BT = Yorkshire × maternal Landrace composite.

al., 2003). Polymorphisms in the leptin gene and leptin receptor have been associated with fatness traits, feed efficiency, and reproduction in pigs (Jiang and Gibson, 1999; Kennes et al., 2001; Chen et al., 2004; Mackowski et al., 2005). Genomic regions associated with age at puberty in the pig (Rohrer et al., 1999; Cassady et al., 2001) and leptin concentrations in mice and humans (Hixson et al., 1999; Harper et al., 2003; Chen et al., 2005) have been identified. Genetic relationships of fat and leptin with age at puberty may provide an alternative means of selecting for decreased age at puberty.

Our initial observations (Wise and Klindt, 2004) indicated that leptin concentrations were associated with the age at puberty in gilts. Using industry-type gilts, the present study examines the genetic correlations among age, BW, body condition as backfat, and leptin concentrations in gilts at first estrus and the association of markers in candidate genes and QTL regions with these traits.

MATERIALS AND METHODS

All animal procedures were reviewed and approved by the US Meat Animal Research Center Animal Care and Use Committee, and procedures for handling gilts complied with those specified in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Animals and Data Collection

Animals born from Yorkshire × maternal Landrace composite (**BT**) were mated to high-lean paternal-line Duroc (n = 12) and Landrace (n = 12) boars obtained from commercial AI studs. The Duroc- and Landrace-sired lines were mated to one another (Duroc × BT and Landrace × BT, respectively) to produce an additional generation of 4-way crossed gilts. Note that the maternal- and paternal-Landrace lines were actually 2 different breeds. Daily detection of estrus consisted of 5 to 6 mature boars (>11 mo of age) placed in an alleyway pen between 2 pens of gilts, during which time herdsmen applied back pressure to gilts within each pen. Estrous detection was initiated at 130 d of age. In addition to age at puberty, backfat (**BFP**) was measured ultrasonically at the first rib, last rib, and last lumbar vertebrae (5 to 8 cm off the midline; Lean-Meater, Renco Corp., Minneapolis, MN), and BW (**BWP**) was recorded at first estrus (Table 1). Blood samples were collected from all females at first estrus by jugular venipuncture in 9-mL Sarstedt (Newton, NC) serum tubes with approximately 150 IU of heparin added. Plasma was obtained via centrifugation at 1,520 × *g*, 4°C for 30 min, and frozen at −20°C for later analysis of leptin concentrations.

Gilts were fed a corn-soybean meal grower ration of 16% protein up to 16 wk of age and 15% protein after 16 wk of age (Klindt et al., 2006). Gilts had ad libitum access to feed and water. Diets were formulated to meet or exceed National Research Council recommendations (NRC, 1988).

Leptin Assay

Leptin concentrations were measured in duplicate in 50 μL of plasma by RIA (DS Labs, Webster, TX) and validated for swine blood (Woodworth et al., 2004). The limit of assay sensitivity was 300 pg/mL. The intra-and interassay CV were 2.6 and 9.1%, respectively. Averages of leptin concentrations at puberty on a logarithmic scale are reported in Table 1.

SNP Marker Development and Genotyping

PCR and Sequencing. Primer pairs for amplification of pig leptin (***LEP***), leptin receptor (***LEPR***), pro-opiomelanocortin (***POMC***), aldo-keto reductases (***AKR1C2*** and ***AKR1C4***), and paired box 5 (***PAX5***) from genomic DNA were designed from porcine sequences deposited in GenBank (U66254, AY845425, TI 861189563 and TI 784738214, DQ474066, DQ494489, and BV680490, respectively; Supplemental Table; http://jas.fass.org/content/vol87/issue2). These genes lie within QTL for age at puberty in the pig (SSC1, *PAX5*; SSC10, *AKR1C2*, and *AKR1C4*; Rohrer et al., 1999) or are genes associated with leptin concentration in human (*LEP*, *LEPR*, and *POMC*). The DNA from 12 breeding boars representing the Duroc and Landrace sire lines was extracted from semen for sequencing and identification of SNP. The PCR was performed in a PTC-200 DNA engine (MJ Research Inc., Watertown, MA) using 0.5 U of HotStarTaq polymerase (Qiagen,

Valencia, CA), 1× of supplied buffer with 1.5 m*M* $MgCl_2$, 200 μ*M* dNTP, 0.8 μ*M* each primer, and 100 ng of genomic DNA in 25-μL reactions. Five microliters of the PCR were electrophoresed in 1.5% agarose gels to determine quality of amplification and the remainder was treated with 0.1 U of exonuclease I (USB Corp., Cleveland, OH) for 30 min, precipitated with isopropanol, and resuspended in water for sequencing on an ABI Prism 3700 DNA analyzer (Applied Biosystems, Foster City, CA; Smith et al., 2000). The PCR products were sequenced directly using the original amplification primers. Resulting sequencing chromatograms were imported into the USMARC database, sequences assembled, and analyzed for polymorphisms using methods described by Nonneman and Rohrer (2003).

SNP Genotyping. The SNP were genotyped using a primer extension assay on the Sequenom MassArray system (San Diego, CA). Ten-microliter PCR reactions contained 10 ng of genomic DNA, 0.25 U HotStarTaq, 1× of supplied buffer with 1.5 m*M* $MgCl_2$, 200 μ*M* dNTP, and 0.4 μ*M* forward and reverse-tailed primers. The primer extension reaction used 0.6 μ*M* of probe primer and was performed according to the manufacturer's recommendations for homogenous mass extension chemistry (Sequenom).

Statistical Analysis

All traits were analyzed for distributional properties before variance component and SNP association analyses. Leptin was found to have a highly skewed distribution in this preliminary analysis and was transformed to log units thereafter.

Initially, multitrait variance components were estimated for each puberty trait using the model

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

with

$$\text{var}\begin{bmatrix}\mathbf{a}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{A}\otimes\mathbf{G} & 0\\ 0 & \mathbf{I}\otimes\mathbf{R}\end{bmatrix},$$

where $\mathbf{y}$ was a vector of phenotypes for each puberty measure (e.g., age at puberty); $\mathbf{b}$ was a vector of fixed farrowing groups and breed of origin percentages for each trait; $\mathbf{a}$ was a vector of random polygenic breeding values for each trait; and $\mathbf{e}$ was a vector of random residuals. Incidence matrices $\mathbf{X}$ and $\mathbf{Z}$ relate phenotypes to combinations of either fixed or random effects, respectively. In the variance structure of $\mathbf{a}$ and $\mathbf{e}$, $\mathbf{A}$ is the numerator relationship matrix, $\mathbf{G}$ is the additive (co)variance matrix, $\mathbf{I}$ is an identity matrix, and $\mathbf{R}$ is the residual (co)variance matrix. All analyses were performed using MTDFREML (Boldman et al., 1995); MTDFREML was allowed to iterate until a convergence criterion of 10^{-10} was obtained. Variance components from this analysis were fixed at these levels for all subsequent SNP association tests.

A multilocus version of GenoProb (Thallman, 2002) was used to check for genotyping errors and to calculate SNP genotypic probabilities for animals that were unsuccessfully genotyped. The average success rate of the marker assays was 91% and ranged from 87 to 95%. Additionally, allelic frequencies of founder groups (Duroc, Landrace, and BT) were predicted for each SNP. Probabilities for each genotype were used as independent variables (regressors) in regressions of puberty measures on SNP additive and dominance effects. For animal j, regressors (row vector $\mathbf{x}_j$) were calculated as

$$\mathbf{x}_j' = \begin{bmatrix}x_A\\ x_D\end{bmatrix}_j = \begin{bmatrix}0 & 1 & 2\\ 0 & 1 & 0\end{bmatrix}\begin{bmatrix}p_{aa}\\ p_{Aa}\\ p_{AA}\end{bmatrix}_j,$$

where p_{aa} (p_{AA}) is the probability that j is homozygous for allele a (A), and p_{Aa} is the probability that j is heterozygous. Resulting regressors x_A and x_D were fitted in the multitrait model for fixed regressions of SNP additive and dominance effects, respectively. Each SNP was initially fitted individually for each trait.

Effects of SNP were tested for significance using an F-test with 2 numerator df. Individual regressions were also tested using a 2-tailed t-test. No adjustments were made for multiple comparisons or multiple tests. After SNP with significant effects were identified individually, they were fitted simultaneously. To determine a final model, the SNP that was the least significant was removed one at a time in a step-down manner, until all remaining SNP were significant at a level of $P = 0.10$ or less.

RESULTS

A description of the gilts evaluated in each birth year × breed type is summarized in Table 1. Age at puberty was earlier ($P = 0.04$), and BFP ($P < 0.01$) and BWP ($P < 0.01$) were both less on average in the 4-way cross gilts. These differences may be due to genetic differences in the crosses or, more likely, simple environmental differences across years. Gilt BWP was approximately 10 kg heavier for Duroc-sired F_1 gilts ($P < 0.01$) than Landrace-sired F_1 gilts. Because age at puberty was not significantly different between the 2, the increase in BW can be attributed to the Duroc line rather than differences in age.

Both BWP and leptin exhibited moderate phenotypic correlations with age at puberty (r = 0.59 and r = −0.52, respectively; $P < 0.001$). The relationship between BFP and age at puberty was less pronounced and differed between the lines; the overall phenotypic correlation was 0.20, but on a within-line basis, the phenotypic correlations were 0.39, 0.38, and −0.18 for

Table 2. Estimates of (co)variance components (±SE) from a 4-trait multivariate model for age at puberty, leptin at puberty, backfat at puberty, and BW at puberty[1,2]

	h^2, r_g, and r_e				
Trait	AP	Lep	BFP	BWP	σ_p^2
AP	0.46 ± 0.078	−0.63 ± 0.097	0.39 ± 0.126	0.65 ± 0.083	642.7
Lep	−0.51 ± 0.057	0.38 ± 0.078	0.12 ± 0.147	−0.22 ± 0.145	1.426
BFP	0.03 ± 0.080	0.21 ± 0.073	0.45 ± 0.069	0.53 ± 0.099	15.47
BWP	0.55 ± 0.056	0.01 ± 0.080	0.34 ± 0.068	0.48 ± 0.072	317.6

[1]Heritability (h^2) estimates are on the diagonals; genetic correlations (r_g) are above the diagonal; and residual correlations (r_e) are below the diagonal; σ_p^2 = phenotypic variance.
[2]AP = age at puberty (d); Lep = leptin at puberty [log(ng/mL)]; BFP = backfat at puberty (mm); BWP = BW at puberty (kg).

the Duroc-sired F_1, Landrace-sired F_1, and 4-way cross gilts, respectively.

All traits were highly heritable with genetic correlations similar in magnitude to the respective phenotypic correlations with age at puberty (Table 2). Both leptin and BWP had slightly greater genetic correlations with age at puberty relative to their phenotypic correlations. A moderate, positive correlation was observed between BWP and BFP. However, the genetic correlation between BFP and age at puberty was not significant.

Polymorphisms were detected in 12 boars from the 4-way cross in *AKR1C2*, *AKR1C4*, *LEP*, *LEPR*, *PAX5*, and *POMC* genes (Table 3). Linkage disequilibrium was low for markers within *AKR* and *LEPR* (r^2 = 0.07 to 0.19) and greater for markers in *POMC* (r^2 = 0.71). Except for SNP in *AKR1C2*, *AKR1C4*, and *LEPR*, the polymorphisms genotyped are assumed to be neutral (no AA change). One SNP in *AKR1C2* changed an isoleucine to phenylalanine; an SNP in *AKR1C4* was located near the splice site in intron 8; and 2 SNP in exon 4 of *LEPR* would result in nonsynonymous amino acid changes (Supplemental Table). The frequencies of the SNP genotyped are shown in Table 3. Whereas other SNP were identified in these sequences, these SNP were chosen for genotyping because of minor allele frequency or ability to design mass extension assays.

In the final model (Table 4), only the polymorphism in *PAX5* had a significant association ($P = 0.036$) with age at puberty with an additive effect of approximately 3.24 d. This same polymorphism was also associated with a significant decrease (in the same direction as age at puberty) in BFP and a slight trend in decreased BWP. Both of these associations are likely the result of the animals being younger at puberty.

A polymorphism in *LEPR* was significantly (P = 0.009) associated with blood leptin concentrations. This polymorphism results in an AA change from isoleucine to leucine. However, the isoleucine coding allele was infrequent (Table 3). No significant associations affecting leptin concentration were identified in the *POMC* or *LEP* genes.

DISCUSSION

Traditionally, reducing age at puberty in farrowing gilts has been a selection goal to realize increased genetic progress by reducing generation interval and to reduce the time and expense of feeding sows before producing a litter (Hixon et al., 1987). Using economic models, Schukken et al. (1994) considered 200 to 220 d of age to be the optimal age for first conception. Other research has shown other desirable gains from selection on age at puberty. In lines divergently selected (1 generation) for age at puberty, early puberty sows farrowed more litters per sow and had more pigs born in each litter (Nelson et al., 1990). In addition, age at puberty is moderately genetically correlated (r = 0.45) with weaning to estrus interval (Sterning et al., 1998). Unfortunately, measurement of age at puberty is labor-intensive and impractical for large-scale facilities. Tools

Table 3. SNP genotyped in resource population

Gene symbol	Map position	USMARC[1] assay ID	Minor allele freq.	Duroc allele freq.	Landrace allele freq.	BT[2] allele freq.	Polymorphic site
AKR1C2	10:127	49422_42	T = 0.07	T < 0.01	T = 0.04	T = 0.13	cggtcacctcWttcctgtact
AKR1C4	10:127	49431_198	C = 0.21	C = 0.04	C = 0.17	C = 0.37	ttcccttcctYycaggttttt
LEP	18:23	27654_595	A = 0.06	A = 0.17	A = 0.00	A = 0.07	agacaagktcRaactgtggcc
LEPR	6:122	49040_157	C = 0.42	C = 0.16	C = 0.17	T = 0.19	gttgttgaaaYggaacttaat
LEPR	6:122	49040_168	A = 0.05	A = 0.13	A = 0.04	A = 0.08	ggaacttaatWyaagtggtac
PAX5	1:97	50422_249	T = 0.42	T = 0.44	T = 0.33	T = 0.44	gttcccaggcYaggggtcgaa
POMC	3:89	50397_39	C = 0.41	T = 0.47	C = 0.38	C = 0.36	ttcgtttctcYgccctttgtc
POMC	3:89	50401_191	C = 0.45	T = 0.44	C = 0.50	C = 0.39	ccagtgagggYtcaggggca

[1]USMARC: US Meat Animal Research Center, Clay Center, NE.
[2]BT = Yorkshire × maternal Landrace composite.

Table 4. Markers with *P*-values of less than 0.10 for puberty traits using a 2 df *F*-test (additive and dominance effects) when fitted simultaneously in a backward selected model

Gene	Assay	Trait[1]	*P*-value	Component[2] ($P < 0.10$)	Genotypic mean[3]	
					Het	aa
PAX5	50422_249	AP	0.036	b_A	−4.22	−6.47
		BFP	0.029	b_A	−0.86	−1.21
		BWP	0.089	b_A	−3.73	−4.22
LEPR	49040_168	Lep	0.009	b_A[4]	0.00	−0.31
AKR1C4	49431_198	Lep	0.059	b_A	0.32	0.43

[1]AP = age at puberty (d); Lep = leptin at puberty [log(ng/mL)]; BFP = backfat at puberty (mm); BWP = BW at puberty (kg).

[2]Components contributing to significance: b_A = additive, b_D = dominance.

[3]Minor allele homozygote = aa = 0; Het = heterozygotic phase average.

[4]Dominance regression not fitted due to confounding; additive effect (b_A) is the difference between the major homozygote mean and the heterozygote mean.

to predict breeding values for age at puberty through genomics or alternative phenotypes would be of great benefit to the producer.

In this study, age at puberty was estimated to be slightly more heritable (0.46) compared with most literature estimates (range 0.25 to 0.55; Lamberson et al., 1991; Johnson et al., 1994; Sterning et al., 1998; Cassady et al., 2002). Leptin at puberty was moderately heritable at 0.38. Literature estimates of leptin concentration were scarce and generally at fixed time points. Cameron et al. (2003) estimated heritability of leptin in Large White pigs between 0.05 and 0.46 during different phases (30 to 90 kg) of a feed test. Generally, greater heritability estimates were observed at the end of the feed test.

The increased genetic correlation (0.65) observed between BWP and age at puberty was not surprising given the range in age at puberty observed in this study. The high negative genetic correlation (−0.63) between age and leptin concentrations at puberty was not expected. Like BWP, this correlation may have been due to a phenotypic decrease in leptin concentrations as animals aged, or these animals may have had decreased concentrations of leptin before reaching puberty. From this genetic correlation, it would appear that leptin may be a useful indicator trait for predicting age at puberty in genetic evaluation programs. However, for a separate study (Klindt et al., 2006), blood samples were taken from a portion of this population (2001 gilts; Duroc × BT, Landrace × BT) at fixed prepubertal ages (128, 149, and 180 d); leptin concentrations obtained on 280 of these gilts were not phenotypically correlated with age at puberty ($r < 0.05$; unpublished data).

The relationship between age, BW, body composition, and attainment of puberty in swine is controversial. It has been postulated that a certain level of body condition is necessary for puberty attainment in gilts (Gaughan et al., 1997), whereas dietary treatments do not seem to affect pubertal age (Patterson et al., 2002). However, gilts with greater growth rate and backfat have shorter weaning to estrus intervals (Tummaruk et al., 2001) and age at puberty is positively genetically correlated with weaning to estrus interval and signs of estrus (Sterning et al., 1998). In reviewing growth and puberty reports, Rauw et al. (1998) noted that gilts with a greater lean percentage had a delayed onset of puberty, and negative genetic correlations have been reported between growth rate and estrus signs at puberty (Rydhmer et al., 1994). Leptin has long been recognized as a putative signal that links metabolic status with the neuroendocrine control of reproduction and puberty in pigs, and attainment of puberty may be related to a metabolic state at a critical period of development rather than a level of body composition (Rozeboom et al., 1995).

Because of the association between leptin concentrations and the attainment of puberty in the gilts in the current study, genes and genomic regions associated with age of puberty and leptin concentrations were targeted to test for associations with these traits. Only 2 reports have identified age at puberty QTL in the pig (Rohrer et al., 1999; Cassady et al., 2001) on chromosomes 1, 7, 8, 10, and 12, and SNP in one of these regions on chromosome 10 are possibly associated (Nonneman et al., 2006) with age at puberty. The QTL for leptin concentrations in humans have been identified in the *POMC* gene, and SNP and *POMC* haplotypes are associated with circulating leptin, body mass index, BW, and body fat (Hixson et al., 1999; Chen et al., 2005). Liefers et al. (2004) identified a polymorphism in *LEPR* associated with leptin concentration in pregnant heifers. Our results indicated a significant association of marker 49040_168 in *LEPR* with leptin concentration at puberty. The same allele identified with leptin in this study (*LEPR*; 49040_168) has been associated with backfat in finishing gilts (Mackowski et al., 2005). The marker associated with age at puberty in this study was in *PAX5* (50422_249). This polymorphism is in an intron, and *PAX5* is not considered a candidate gene for age at puberty. However, it is located under a QTL for age at puberty identified by Rohrer et al. (1999). The additive effect detected in this study was less than

that reported by Rohrer et al. (1999) for the QTL (9.25 d). The QTL discovery population in Rohrer et al. (1999) was developed from Meishan and White Composite founders. Because Meishans typically have very early puberties, the additive effect estimated for the QTL may have been greater. It is reasonable that the additive effect in this study would be smaller. This was the only marker in this QTL region genotyped in this population; this region warrants further fine-mapping to detect SNP with stronger associations. Note that neither of these associated SNP was corrected for the multiple testing that occurred in this project. Therefore, their effect in a separate population should be validated before they are used in breeding programs.

In conclusion, gilts that had delayed puberty were heavier and were as fat as gilts that had an earlier pubertal age. Circulating leptin concentrations were less in animals exhibiting delayed puberty. Because the heritability of age at puberty is relatively high, producers should exercise caution when selecting replacements from gilts expressing late puberty. This study indicates that using leptin or other physiological measures for selecting animals for decreased age at puberty is not as effective as direct measurement. However, predictive genetic markers may be more effective due to the high labor cost of measuring age at puberty.

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